Serologic anti-GP2 antibodies are associated with genetic polymorphisms, fibrostenosis, and need for surgical resection in crohn's disease

Degenhardt, Frauke ; Dirmeier, Andrea ; Lopez, Rocio ; Lang, Sylvia ; Kunst, Claudia ; Roggenbuck, Dirk ; Reinhold, Dirk ; Szymczak, Silke ; Rogler, Gerhard ; Klebl, Frank ; Franke, Andre ; Rieder, Florian

Abstract: BACKGROUND The presentation of Crohn's disease (CD) is heterogeneous and often leads to serious complications and need for surgery. We tested serum anti-zymogen granule glycoprotein 2 (GP2) antibodies, including its novel isoform alpha, for association with genetic variants, diagnosis, disease stratification, and prediction of CD courses in a combined cross-sectional and cohort study. METHODS Serum samples of 303 CD, 108 ulcerative colitis, 72 other inflammatory gastrointestinal diseases, and 206 controls without predominant gastrointestinal diseases controls (HC) were tested for the presence of Anti-GP2 and Anti-Saccharomyces cervisiae (ASCA) by enzyme-linked immunosorbent assay. Genetic analysis was performed using the Illumina Immunochip. RESULTS GP2 IgA and IgG had the highest discriminatory capability for CD versus ulcerative colitis and CD versus inflammatory gastrointestinal diseases. We identified an association of GP2 IgA and IgG each with 5 distinct single-nucleotide polymorphisms. Levels of anti-GP2 IgG were moderately associated with ileal disease location. Interestingly, both, anti-GP2 IgA and IgG were exclusively associated with the occurrence of stenosis and need for surgery, independently of disease location, but not with fistulizing CD, early disease onset or disease activity. ASCA IgG and IgA were qualitatively and quantitatively linked to CD, CD complications, and need for surgery. Increased levels of ASCA IgG and IgA and positivity for ASCA IgG, but neither levels nor positivity for GP2 IgG or IgA were predictive of the earlier occurrence of complications or surgery. CONCLUSIONS Anti-GP2 antibodies may aid as a tool for diagnosis and differentiation of CD and could indicate a more complicated CD course.

DOI: https://doi.org/10.1097/MIB.0000000000000936

Serologic Anti-GP2 Antibodies Are Associated with Genetic Polymorphisms, Fibrostenosis, and Need for Surgical Resection in Crohn's Disease

Frauke Degenhardt, Andrea Dirmeier,† Rocio Lopez, MS,‡ Sylvia Lang,§ Claudia Kunst, PhD,† Dirk Roggenbuck, MD, PhD,‖ Dirk Reinhold, MD, PhD,¶ Silke Szymczak, PhD,*,** Gerhard Rogler, MD, PhD,§,†† Frank Klebl, MD, PhD,† Andre Franke, PhD,* and Florian Rieder, MD†,‡‡*

Background: The presentation of Crohn's disease (CD) is heterogeneous and often leads to serious complications and need for surgery. We tested serum anti-zymogen granule glycoprotein 2 (GP2) antibodies, including its novel isoform alpha, for association with genetic variants, diagnosis, disease stratification, and prediction of CD courses in a combined cross-sectional and cohort study.

Methods: Serum samples of 303 CD, 108 ulcerative colitis, 72 other inflammatory gastrointestinal diseases, and 206 controls without predominant gastrointestinal diseases controls (HC) were tested for the presence of Anti-GP2 and Anti-*Saccharomyces cervisiae* (ASCA) by enzyme-linked immunosorbent assay. Genetic analysis was performed using the Illumina Immunochip.

Results: GP2 IgA and IgG had the highest discriminatory capability for CD versus ulcerative colitis and CD versus inflammatory gastrointestinal diseases. We identified an association of GP2 IgA and IgG each with 5 distinct single-nucleotide polymorphisms. Levels of anti-GP2 IgG were moderately associated with ileal disease location. Interestingly, both, anti-GP2 IgA and IgG were exclusively associated with the occurrence of stenosis and need for surgery, independently of disease location, but not with fistulizing CD, early disease onset or disease activity. ASCA IgG and IgA were qualitatively and quantitatively linked to CD, CD complications, and need for surgery. Increased levels of ASCA IgG and IgA and positivity for ASCA IgG, but neither levels nor positivity for GP2 IgG or IgA were predictive of the earlier occurrence of complications or surgery.

Conclusions: Anti-GP2 antibodies may aid as a tool for diagnosis and differentiation of CD and could indicate a more complicated CD course.

(Inflamm Bowel Dis 2016;22:2648–2657)

Key Words: serum antibody, fibrosis, stricture

Inflammatory bowel disease (IBD) commonly suffers from a delay in time from the first occurrence of symptoms to diagnosis.[1] Once the diagnosis of IBD is made, its subcategorization into Crohn's disease (CD) or ulcerative colitis (UC) is critical for determining the optimal treatment strategy.[2,3] Development of complicated disease behavior, for the purpose of our study defined as the occurrence of internal fistulae or stenoses, is a frequent event during the disease course and has important clinical implications.[4,5] New tools are necessary at a clinical level to aid in the diagnosis and differentiation of IBD or to screen for individ-

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.ibdjournal.org).

Received for publication July 7, 2016; Accepted August 4, 2016.

From the *Institute of Clinical Molecular Biology, Christian-Albrechts-University of Kiel, Kiel, Germany; †Department of Internal Medicine I, University Medical Center Regensburg, Regensburg, Germany; ‡Department of Quantitative Health Sciences, Cleveland Clinic Foundation, Cleveland, Ohio; §Division of Gastroenterology and Hepatology, University Hospital Zurich, Zurich, Switzerland; ‖Faculty of Science, Brandenburg University of Technology Cottbus-Senftenberg, Senftenberg, Germany; ¶Institute of Molecular and Clinical Immunology, Otto-von-Guericke University Magdeburg, Magdeburg, Germany; **Institute of Medical Informatics and Statistics, Christian-Albrechts-University of Kiel, Kiel, Germany; ††Zurich Center for Integrative Human Physiology, University of Zurich, Zurich, Switzerland; and ‡‡Department of Gastroenterology, Hepatology and Nutrition, Cleveland Clinic Foundation, Cleveland, Ohio.

Supported by the Bundesministerium für Bildung und Forschung (German Ministry of Education and Research) and Kompetenznetz chronisch entzündliche Darmerkrankungen (Competence network "Inflammatory Bowel Disease").

A. Franke and F. Rieder contributed equally.

Author disclosures are available in the Acknowledgments.

Address correspondence to: Florian Rieder, MD, Department of Gastroenterology, Hepatology and Nutrition, Digestive Disease Institute, Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, OH 44195 (e-mail: riederf@ccf.org).

DOI 10.1097/MIB.0000000000000936

Published online 4 October 2016.

uals particularly susceptible to the development of complicated CD behavior leading to early surgery—making an early or more aggressive therapeutical intervention possible.

Over the past decade, serological markers for IBD, such as anti-*Saccharomyces cervisiae* (ASCA) have been the focus of extensive investigation, not only for the diagnosis and differentiation of IBD but also for disease stratification.[6,7] More recently, additional serum antibodies have been reported in patients with CD.[8–14] Although ASCA is still the serologic markers with the highest accuracy, the overall limited precision of the existing markers restricts their use in clinical practice making testing of novel markers and panels of markers necessary.

Pancreatic autoantibodies (PAB) are known to be associated with CD, being present in about up to 42% of patients, but not with UC or healthy controls.[15–17] Recently glycoprotein 2 (GP2) has been identified as the major target antigen of PAB.[18] Since then, few studies investigated the value of anti-GP2 antibodies for diagnosis and differential diagnosis of IBD as well as the link with disease courses.[19–26]

Despite this information, several items remain unclear: (1) Information is lacking comparing IBD to non-IBDs of the intestine and hence allowing the differentiation of a CD-specific effect from a nonspecific inflammatory phenotype. (2) Only a single very recent publication investigated predictive capabilities for complicated CD.[21] (3) The published studies use isotype beta of anti-GP2 and no information is available on the novel isotype alpha of anti-GP2. (4) Essentially, no information is available about a possible genetic susceptibility to the formation of anti-GP2 antibodies. This study was designed to fill these knowledge gaps by evaluating the ability of anti-GP2 IgG and IgA for distinct objectives: diagnosis and differentiation of CD, including comparison with other inflammatory gastrointestinal diseases (OGD), association with and prediction of complicated disease behavior, comparison to an available gold standard and association with genetic variants.

MATERIALS AND METHODS

Patient Population

Serum samples were obtained from 303 patients with CD seen at the IBD center of the Department of Internal Medicine I, University Medical Center Regensburg, Regensburg, Germany. The sera belong to the serum repository of the German Competence Network IBD. CD was diagnosed based on clinical, radiographic, endoscopic, and histopathologic criteria according to the European Crohn's and Colitis organization clinical consensus guidelines.[3] Patient characteristics are shown in Table 1. All CD inpatients and outpatients seen at our clinic between 2000 and 2006 were asked to participate in the serum repository. Patients with indeterminate colitis were excluded because of their low number, which made a meaningful analysis impossible.

The control groups consisted of 386 individuals: 108 patients with UC, 72 patients with OGD, including non-IBD gastrointestinal inflammation such as diverticulitis/-osis, infectious colitis, pseudomembranous colitis, chemotherapy-induced colitis, intestinal vasculitis as well as patients with liver cirrhosis, a disease known to be accompanied by increased intestinal permeability, and 206 controls (non-IBD/GI) with no IBD, no gastrointestinal disorder (e.g., hypertension or diabetes mellitus only), or no apparent disease. The patients with liver cirrhosis were included as serologic antibodies associated with CD were found to be elevated in this patient population.[27,28] The diagnosis of UC was based on clinical, radiographic, endoscopic, and histopathologic criteria as summarized in the European Crohn's and Colitis organization clinical consensus guidelines.[2]

We additionally performed a cohort study among adult patients with CD. Only patients without any complications, defined as fistula or stenoses, and no CD-related surgery before or within 20 days of sample procurement (totally naive population [n = 70]; see Table 1, Supplemental Digital Content 1, http://links.lww.com/IBD/B353) were included. Patients were excluded if the time from serum procurement to event was ≤20 days or if they had a pure inflammatory disease course with a follow-up of less than 3 years. This patient cohort represents a subgroup of the above reported cross-sectional study. Follow-up for a particular patient was terminated at the time of the last available patient encounter.

Clinical data including age at and time of diagnosis, body mass index, gender, date of sample procurement, date, type and frequency of complications and surgery, disease location, disease status, and medications were obtained or updated, respectively, for each time point of sample procurement separately by the treating physician of the IBD unit. Once collected, data were transferred and stored in a securely coded pseudonymized database for analysis. Disease activity was determined as a yes/no by the treating physician respecting all criteria included in the CDAI. A Crohn's disease activity index (CDAI) cutoff of 150 was used. Before finishing data collection, all patient charts and the database were reviewed and updated for the data points mentioned above.

Phenotypical Characteristics of Patients with IBD

Patients with CD were assigned a behavioral phenotype according to the Montreal Classification.[29] For the purpose of this study, complicated disease behavior in patients with CD was defined as the occurrence of fistula or stenoses before or during follow-up. For the classification of internal penetrating versus stricturing disease, either cross-sectional imaging was required or the diagnosis was made post surgical resection through histopathology. Furthermore, we examined the need for CD-related surgery during the follow-up period. Patients undergoing CD-associated abdominal surgery were separated from CD-related perianal surgery, such as perianal abscess drainage or perianal fistula treatment. Early disease onset was defined according to the Montreal classification as disease onset <40 years of age.

Serological Analysis

After the blood draw, serum was separated by centrifugation and kept frozen at −80°C until use. Anti-GP2 IgG and IgA as

TABLE 1. Patient Characteristics

Factor	CD (N = 303)	UC (N = 108)	OGD (N = 72)	Non-IBD/GI (N = 206)	*P*
Female,[a] n (%)	160 (52.8)	43 (39.8)[b]	26 (36.1)[b]	120 (58.8)[c,d]	***<0.001***[e]
Age at sample procurement,[a] mean ± SD	36.1 ± 12.5[b,c,d]	40.2 ± 12.8[b,d,f]	60.3 ± 13.8 [b,c,f]	45.9 ± 15.4 [c,d,f]	***<0.001***[g]
Body mass index,[a] mean ± SD	23.6 ± 5.4[b,d]	24.8 ± 5.1	26.8 ± 5.7[f]	26.4 ± 6.6[f]	***<0.001***[g]
Age at diagnosis,[a] mean ± SD	28.5 ± 12.3[c]	32.9 ± 13.1[f]	—	—	***0.002***[g]
Disease duration,[a] median (Q1, Q3), mo	68.7 (15.7, 138.0)	60.8 (22.9, 148.1)	—	—	0.84[h]
Location (nonexclusive)					
Upper GI tract,[a] n (%)	31 (10.4)	—	—	—	
Jejunum, prox. ileum,[a] n (%)	35 (11.7)	—	—	—	
Ileocecal,[a] n (%)	93 (30.7)	—	—	—	
Colon (w/o cecum),[a] n (%)	43 (14.2)	—	—	—	
Ileum and colon,[a] n (%)	166 (55.0)	—	—	—	
Rectum,[a] n (%)	96 (31.8)	—	—	—	
Any ileum involvement,[a] n (%)	259 (85.5)	—	—	—	
Montreal classification,[a] n (%)					
B1	69 (22.8)	—	—	—	
B1p	21 (6.9)	—	—	—	
B2	86 (28.4)	—	—	—	
B2p	18 (5.9)	—	—	—	
B3	76 (25.1)	—	—	—	
B3p	33 (10.9)	—	—	—	
Medications at sample procurement[a]					
Infliximab	1 (0.33)	—	—	—	
Steroids	150 (49.7)	—	—	—	
Immunosuppressant	176 (58.3)	—	—	—	
Medications during follow-up[a]					
Infliximab	6 (2.0)	—	—	—	
Steroids	172 (57.0)	—	—	—	
Immunosuppressant	201 (66.6)	—	—	—	
Surgery,[a] n (%)	219 (72.3)	—	—	—	

A significance level of 0.008 was used for pairwise ad hoc comparisons. Significant values are depicted in bold and italic.
[a]Data not available for all subjects. Missing values: female = 2, age at sample procurement = 2, body mass index = 19, upper GI-tract = 4, jejunum, prox. ileum = 4, ileocecal = 4, ileum and colon = 1, rectum = 1, ileum involvement = 1, medications = 1.
[b]Significantly different from non-IBD/GI.
[c]Significantly different from UC.
[d]Significantly different from OGD.
[e]*P*-values = Pearson's chi-square test.
[f]Significantly different from CD.
[g]*P*-values = analysis of variance.
[h]*P*-values = Kruskal–Wallis test.

well as ASCA IgG and IgA were measured in the serum samples by enzyme-linked immunosorbent assay in a blinded fashion without the knowledge of the patients' diagnosis or other clinical information and following the manufacturers' conditions (GA Generic Assays, Dahlewitz, Berlin, Germany) as reported previously.[30] The optical density obtained is directly proportional to the amount of antibody. Results were expressed as arbitrary units, provided by the manufacturer of the enzyme-linked immunosorbent assay kits, which is a value relative to a standard serum pool derived from patients with well-characterized disease found to react to the above-mentioned antigens.

Two different isoforms of GP2 are synthesized in the pancreas, a larger isoform (termed alpha) and a shorter isoform (termed beta).[31] GP2 IgA and IgG assays are based on recombinant human GP2 isoforms alpha and beta expressed in *Spodoptera frugiperda 9* cells as solid-phase antigen.[30] The anti-GP2 IgA and IgG enzyme-linked immunosorbent assays displayed an interassay variability of 5.0% each for a serum of 26.9 and 18.5 U/mL,

respectively.[32] The functional assay sensitivity representing the lowest antibody concentration with a coefficient of variation of smaller than 20% was determined at 2.4 and 1.8 U/mL for anti-GP2 IgG and anti-GP2 IgA, respectively.[33] For ASCA IgG and IgA, the interassay coefficient of variation was 5.9% for a sample containing 72 U/mL and 1.8% for a sample containing 81 U/mL of ASCA IgA and ASCA IgG, respectively. Antinuclear antibody, anti-PR2 antibody, and anti-myeloperoxidase antibody were measured in all serum samples following the manufacturers' instructions (GA Generic Assays).

Accuracy Analysis and Cutoff Values

For the purpose of our study, we evaluated the antibody markers for distinct clinical objectives: presence in CD, diagnosis of CD, and differentiation between CD and UC, OGD and non-IBD/GI, quantitative and qualitative association or prediction of the serum antibodies with disease phenotypes, namely the occurrence of complications, IBD-related surgery, early disease onset and disease location, and association of anti-GP2 level with genetic variants. Receiver operating characteristics (ROC) curves were calculated by plotting sensitivity versus (1-specificity) for each individual marker. Based on the ROC curves, cutoff values were determined for each clinical scenario, optimizing specificity.

Genotyping and Quality Control

Genotyping and quality control can be found in supplemental methods (see Fig. 2, Supplemental Digital Content 2, http://links.lww.com/IBD/B354).

Statistical Analysis

Data are presented as mean ± SD, median (25th, 75th percentiles), or n (%). Univariate analysis was performed to assess differences between the disease groups. Analysis of variance or the nonparametric Kruskal–Wallis tests were used for continuous or ordinal variables, and Pearson's chi-square tests were used for categorical factors. In addition, ROC curves were constructed and the areas under the curves (AUCs) with their corresponding 95% confidence intervals (CIs) were estimated. Using the cutoff values for each marker as suggested by the ROC curves, antibody positivity was determined for each subject. Sensitivity, specificity, and positive and negative predictive values were estimated to assess the validity of each marker in diagnosing CD. We assessed a possible association of anti-GP2 and anti-ASCA serum levels with available variables. We tested for association with body mass index (categorized in <25 and ≥ 25) and gender using an unpaired 2-sided t test, assuming equal variance and with age (categorized into the intervals 0–20, 21–40, 41–60, and above 60) using analysis of variance and R version 3.0.2 (R Institute for Statistical Computing, Vienna, Austria). A time-to-event analysis was performed to assess whether antibodies are associated with occurrence of first complication and/or surgery. Time of follow-up was defined as months between the time of antibody levels determination and the occurrence of first event or last record of patient history if no events were observed. Kaplan–Meier plots were constructed and log-rank tests were used to assess differences in event-free rates between groups. In addition, univariable and multivariable Cox proportional hazard models were constructed, and the hazard ratios (HRs) and 95% CIs for each marker were estimated. For other analyses, SAS version 9.2 (The SAS Institute, Cary, NC) and R version 3.0.1 were used. For all statistical tests, a P-value of <0.05 was considered statistically significant.

Analysis Genome-Wide Association Study

Genome-wide association (GWA) study can be found in supplemental methods Fig. 2, Supplemental Digital Content 2, http://links.lww.com/IBD/B354.

Ethical Considerations

Signed informed consent was obtained from all participants. The study was approved by the ethics committee of the University of Regensburg.

RESULTS

Clinical Phenotypes of the Population

A total of 689 unrelated participants were eligible for analysis (Table 1). The largest differences between the cohorts were found for age at sample procurement. Two hundred thirteen patients with CD had a complication, defined as fistula or stricture, before or at the time of sample procurement. After a median follow-up, after sample procurement of 59.8 months, the total number of patients with CD who experienced a disease complication increased to 230. Ninety-one of the patients with complicated disease behavior had both, fistulae and stenoses. Two hundred nineteen patients with CD already had IBD-related surgery at the time of sample procurement. Eleven additional patients with CD had surgery during follow-up.

Presence of Anti-GP2 Antibodies in CD and Use for Diagnosis and Differentiation

Levels of anti-GP2 isoform beta antibodies were significantly higher in CD than in all control groups (Table 2; $P < 0.001$). Since the cutoff values and discriminatory capability of the novel anti-GP2 antibodies have not been uniformly standardized in patients with CD, we initially tested the immune responses of each individual marker for 4 different settings: Differentiation of CD versus non-CD, CD versus UC, CD versus OGD, and CD versus non-IBD/GI. For this purpose, ROC curves were generated for each individual marker (Fig. 1). GP2 IgA and GP2 IgG isoform beta were the markers with the highest discriminatory accuracy with AUC values between 0.67 and 0.8 (see Table 2, Supplemental Digital Content 3, http://links.lww.com/IBD/B355). Based on the ROC curves, optimal cutoff values were determined. The proportion of the positive markers in the different cohorts is shown in Table 2. 25.4% and 19.8% of the participants were positive for anti-GP2 IgA or IgG isoform beta, respectively, which was higher than all other control groups. Based on the cutoff values, we tested the validity of each single marker for the conditions CD versus all other

TABLE 2. Marker Distribution

Factor	CD (N = 303)	UC (N = 108)	OGD (N = 72)	Non-IBD/GI (N = 206)	*P*
Marker distribution					
GP2 IgA isoform beta	5.6 (2.1–15.6)[a,b,c]	1.2 (1.00–2.2)[d]	1.8 (1.00–2.9)[d]	1.5 (1.00–2.6)[d]	***<0.001***[e]
GP2 IgG isoform beta	6.6 (4.1–12.8)[a,b,c]	3.5 (2.2–7.4)[d]	3.5 (2.2–6.7)[d]	4.2 (2.9–7.1)[d]	***<0.001***[e]
ASCA IgA	10.9 (2.9–51.2)	15.8 (11.7–20.0)	17.8 (14.0–32.9)	17.4 (11.0–27.2)	***0.013***[e]
ASCA IgG	17.7 (5.2–52.9)[a,c]	6.4 (4.1–12.6)[d]	11.4 (4.8–19.2)	7.9 (3.5–15.1)[d]	***<0.001***[e]
Marker positivity					
GP2 IgA isoform beta >15	77 (25.4)[a,b,c]	0 (0.0)[d]	0 (0.0)[d]	3 (1.5)[d]	***<0.001***[f]
GP2 IgG isoform beta >15	60 (19.8)[a,c]	6 (5.6)[d]	6 (8.3)	11 (5.3)[d]	***<0.001***[f]
ASCA IgA >20	132 (43.6)[a]	27 (25.0)[b,d]	32 (44.4)[a]	73 (35.4)	***0.004***[f]
ASCA IgG >20	132 (43.6)[a]	27 (25.0)[b,d]	32 (44.4)[a]	73 (35.4)	***0.004***[f]
No. positive markers					***<0.001***[e]
0	133 (43.9)[a,c]	77 (71.3)[d]	40 (55.6)	129 (62.6)[d]	
1	33 (10.9)	4 (3.7)	0 (0.0)	4 (1.9)	
2	66 (21.8)	25 (23.1)	26 (36.1)	64 (31.1)	
3	48 (15.8)	2 (1.9)	6 (8.3)	8 (3.9)	
4	23 (7.6)	0 (0.0)	0 (0.0)	1 (0.49)	

Values presented as Median (P25, P75) or n (%). A significance level of 0.008 was used for pairwise ad hoc comparisons. Significant values are depicted in bold and italic.
[a]Significantly different from UC.
[b]Significantly different from OGD.
[c]Significantly different from non-IBD/GI.
[d]Significantly different from CD.
[e]*P*-values = Kruskal–Wallis test.
[f]*P*-values = Pearson's chi-square test.

groups, CD versus UC, CD versus OGD, and CD versus non-IBD/GI (see Table 3, Supplemental Digital Content 4, http://links.lww.com/IBD/B356). Anti-GP2 isotype 4 had a high specificity and positive predictive value and low sensitivity and negative predictive value for all tested conditions.

As a control, we evaluated the so far most accurate marker associated with CD ASCA IgG and IgA in our cohort. Although both ASCA antibody levels were higher in CD than in UC and non-IBD/GI, the frequency was only different compared with the UC subjects. Anti-GP2 isotype 4 antibodies had a higher AUC and specificity for all tested conditions. Next, we assessed a possible overlap of ASCA and anti-GP2 isotype 4 in our patients with CD. The Venn diagram can be seen online in Fig. 1, Supplemental Digital Content 5, http://links.lww.com/IBD/B357, showing positivity of these markers in distinct patient populations as well as an overlap between anti-GP IgG and IgA as well as anti-GP2 and ASCA. We also separated the OGD group into subjects with liver cirrhosis and those without for analysis of marker expression. This information can be found in Table 4, Supplemental Digital Content 6, http://links.lww.com/IBD/B358.

Antibody Response and Association with Disease Phenotypes

The response of each individual marker was tested separately for association with complicated disease behavior, need for surgery, ileal disease location, clinically active disease and CRP values. Since this area is less explored, we additionally evaluated the novel isoform alpha of anti-GP2 in this setting. A Venn diagram depicting overlap for positivity of anti-GP2 can be found in Fig. 2, Supplemental Digital Content 7, http://links.lww.com/IBD/B359. The univariate analysis for association with disease phenotypes can be found in Table 5, Supplemental Digital Content 8, http://links.lww.com/IBD/B360.

In multivariate analyses, taking into account early disease onset and ileal involvement as potential confounder, we found an association of anti-GP2 IgG isoform alpha and beta levels with fibrostenosis and anti-GP2 IgG isoform alpha with need for surgery (Table 3). Positivity for GP2 IgG and IgA isoform alpha was linked to a higher risk for complications, fibrostenosis, and need for surgical intervention but not fistulizing disease. Positivity for GP2 IgG isoform beta was associated with higher risk for complications, fistulae, stenoses, and surgery, whereas GP2 IgA isoform beta was linked to higher risk for fistulae and surgery. Next, we evaluated the discriminatory ability of anti-GP2 antibodies for disease phenotypes. GP2 IgG and A Isoforms alpha and beta had an AUC of 0.6 to 0.645 for its association with fibrostenosis and surgery. The discriminatory capability was not different from ASCA IgG. The addition of ASCA IgG to GP2 IgG or IgA of either isoform did not increase its discriminatory abilities (data not shown).

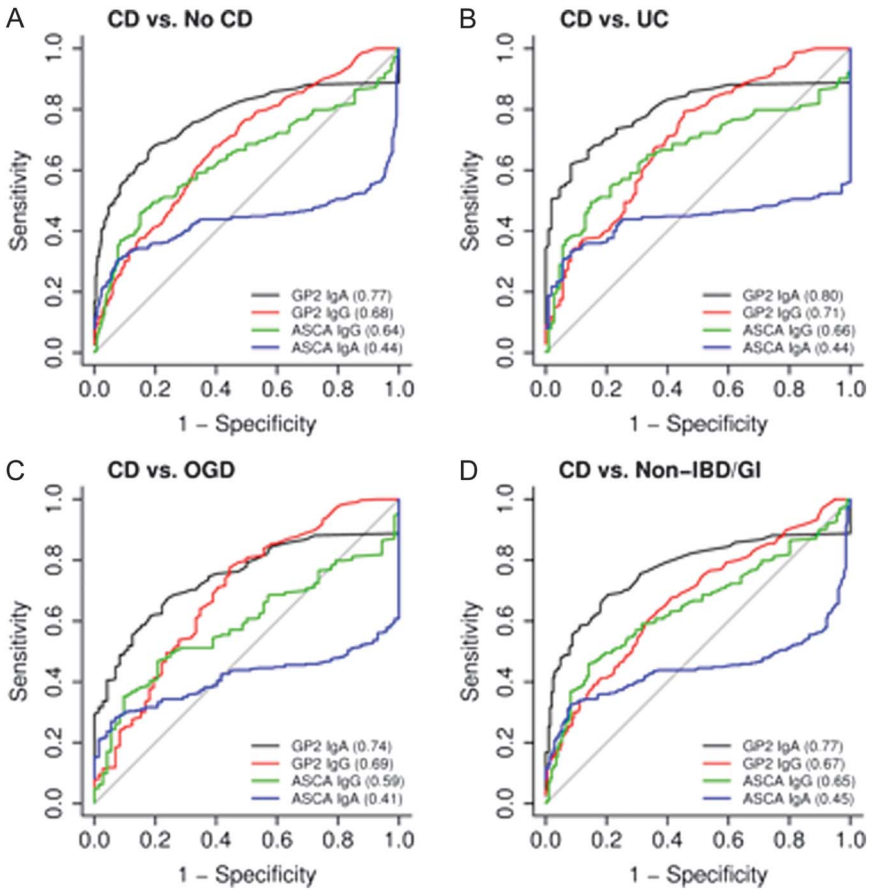

FIGURE 1. ROC for diagnosis and differentiation of CD. A, CS versus non-CD; (B) CD versus UC; (C) CD versus OGD; (D) CD versus non-IBD/GI controls. The plots for anti-GP2 refer to isoform beta.

As previously described,[34] ASCA IgG and ASCA IgA were associated with the occurrence of any complication, fistulae, strictures, need for surgery, ileal involvement, and early disease onset. After correction for ileal disease, location and early disease onset ASCA IgG and IgA remained associated with any complication, fistula, stricture, and need for surgery (Table 3).

Antibody Response and Prediction of Disease Progression

Predictive information for anti-GP2 antibodies is still limited.[21] To obtain prospective information, we performed a cohort study using a subgroup of 70 patients with a purely inflammatory phenotype at time of sample procurement (see Table 1, Supplemental Digital Content 1, http://links.lww.com/IBD/B353). Sera were taken close to diagnosis (median 11.1 months, P25 1.8; P75 50.4) and both isoforms of anti-GP2 were determined. The median follow-up in the complication and surgery-naive group was 59.8 months. Among the naive patients studied, 24.3% experienced any complication during follow-up (11.4% fistulae, 17.1% stenoses). 15.7% had to undergo CD-related surgery during the observation period. The predictive subgroup was compared to the remainder of the included CD subjects (complication at or before sample procurement). There was no difference in demographics, disease characteristics, medication intake, or antibody levels or positivity, with the exception of GP2 isoform 4 IgA with a slightly higher level and frequency in the complication at or before sample procurement group (52.6% positive versus 35.7% positive, $P = 0.013$, data not shown).

In the naive patients progressing to a first event, defined as fistula, stenosis, or IBD-related surgery (composite endpoint), the frequency of antibody positivity or antibody levels of anti-GP2 IgG or IgA isoforms alpha and beta were no different from the naive patients not progressing to a more complicated disease course (see Table 6, Supplemental Digital Content 9, http://links.lww.com/IBD/B361). The same was true for looking at progression to stricture and/or fistulizing disease or strictures or fistulas or need for surgery separately (data not shown).

As a positive control, we used ASCA IgG and IgA. The frequency of antibody positivity or antibody levels for both were higher in the patients showing a faster progression to fistula, stenosis, or IBD-related surgery (composite endpoint; see Table 6, Supplemental Digital Content 9, http://links.lww.com/IBD/B361) as previously reported using a different ASCA assay.[35] Levels

TABLE 3. Odds Ratios for the Association of Markers with Disease Phenotypes Adjusted for Early Disease Onset and Ileum Involvement

	Any Complication		Fistula		Stenosis		Surgery	
Factor	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Levels								
GP2 IgA iso alpha	1.03 (0.96–1.1)	0.39	1.04 (0.98–1.09)	0.2	1.09 (1.00–1.2)	0.057	1.1 (0.97–1.2)	0.13
GP2 IgG iso alpha	1.07 (0.99–1.2)	0.079	1.01 (0.97–1.04)	0.66	1.07 (1.01–1.1)	***0.016***	1.1 (1.01–1.2)	***0.035***
GP2 IgA iso beta[a]	3.5 (0.75–16.6)	0.11	1.3 (0.92–1.9)	0.12	1.8 (0.95–3.6)	0.071	2.4 (0.85–7.0)	0.098
GP2 IgG iso beta[a]	1.1 (0.99–1.3)	0.065	1.02 (0.98–1.06)	0.31	1.08 (1.02–1.2)	***0.014***	1.08 (1.00–1.2)	0.054
ASCA IgA	1.06 (1.01–1.1)	***0.017***	1.03 (1.01–1.06)	***0.021***	1.04 (1.01–1.08)	***0.008***	1.1 (1.05–1.2)	***<0.001***
ASCA IgG	1.2 (1.08–1.3)	***<0.001***	1.06 (0.99–1.1)	0.083	1.1 (1.05–1.2)	***0.001***	1.2 (1.08–1.3)	***<0.001***
Positvity								
GP2 IgA iso alpha ≥2.6	2.0 (1.1–3.5)	***0.018***	1.6 (0.98–2.7)	0.058	2.1 (1.2–3.4)	**0.005**	3.0 (1.8–5.2)	**<0.001**
GP2 IgG iso alpha ≥7.3	2.2 (1.2–3.9)	***0.008***	1.4 (0.88–2.2)	0.15	2.0 (1.2–3.2)	***0.006***	2.8 (1.6–4.8)	***<0.001***
GP2 IgA iso beta ≥1.3[a]	1.6 (0.90–2.7)	0.12	1.9 (1.2–3.1)	***0.006***	1.5 (0.96–2.5)	0.071	2.5 (1.5–4.3)	***<0.001***
GP2 IgG iso beta ≥1.7[a]	2.5 (1.4–4.4)	***0.002***	2.0 (1.2–3.4)	***0.009***	1.7 (1.02–2.9)	***0.042***	2.8 (1.6–4.8)	***<0.001***
ASCA IgA ≥5.5	5.3 (2.9–9.8)	***<0.001***	2.3 (1.4–3.9)	***0.001***	3.5 (2.1–5.8)	***<0.001***	5.7 (3.2–10.2)	***<0.001***
ASCA IgG ≥25.2	4.0 (2.1–7.7)	***<0.001***	1.8 (1.1–3.0)	***0.014***	2.7 (1.6–4.5)	***<0.001***	4.5 (2.4–8.4)	***<0.001***

ORs for marker levels correspond to a 10-unit increase in the markers. Significant values are depicted in bold and italic.
[a]Data not available for all subjects. Missing values: GP2 isoform beta IgA = 1, GP2 isoform beta IgG = 1.
CI, confidence interval; iso, isoform; OR, odds ratio.

and frequency for ASCA IgG were higher in patients with a faster progression to fistula and/or stenosis. Levels of ASCA IgA were elevated in patients with a faster progression to surgery (HR 1.08 [1.02, 1.1]) and positivity for ASCA IgG was higher in patients with faster progression to surgical intervention (HR 3.7 [1.1, 12.6]). Examples for survival curves for anti-GP2 isoform beta and ASCA can be found in Fig. 3, Supplemental Digital Content 10, http://links.lww.com/IBD/B362.

We tested whether the use of steroids or immunosuppressants at the time of sample procurement could influence the marker levels or positivity. At large, there was no association of medication intake and serologic marker expression with the exception of a slightly lower frequency (but not level) of GP2 isoform 1 IgA in patients taking corticosteroids (66.7% versus 41%, $P = 0.035$, data not shown). Since the formation of anti-GP2 antibodies could indicate an autoimmune phenomenon, we measured all serum samples for antinuclear antibody, anti-PR2 antibody, and anti-myeloperoxidase antibody and assessed their ability for diagnosis, discrimination, and association or prediction of disease phenotypes, but none of those markers revealed any link (data not shown).

Genotype–Serotype Association

Serologic antibodies linked to CD have been shown to be familial traits,[36] indicating that genetic variants could have an influence on the expression of serologic markers. To investigate possible genetic variants linked to the levels of anti-GP2 antibodies, we performed a GWA analysis on genotypes using the Illumina Immunochip genotyping array. For the analysis of anti-GP2 IgA and anti-GP2 IgG, a total of 519 samples were available. Since isotype alpha was only available for the CD cohort, we used isotype beta for the genetic analysis. The analysis had an inflation factor of 1.00 (GP2 IgA) and 1.02 (GP2 IgG). The Manhattan quantile–quantile of all analyses can be found in Figs. 4–6, Supplemental Digital Content 11, http://links.lww.com/IBD/B363. Owning to the limited sample size, we lowered the *P*-value threshold to a level of 10^{-4}. In total, 7 variants were selected for GP2 IgA and 5 for GP2 IgG. No variants overlap. The variant with the lowest *P*-value across all chromosomes is located on chromosome 11 at position 76334404 with a nominally significant *P*-value of 2.59×10^{-6} (beta = 0.194, 95% CI [0.114, 0.274]). This variant is located approximately 34 kB downstream of *LRCCR32* with has been described, among others, in the context of CD and UC. In addition, we performed an LD-based clumping to identify "local best single-nucleotide polymorphisms (SNPs)" as described in the Materials and Methods section. The local best SNPs were paired with imputed markers that had a lower association *P*-value within the same region. These variants, as well as the local effect of these variants and regional plots, are shown in Table 4, Tables 7 and 8, Supplemental Digital Content 12, http://links.lww.com/IBD/B364 and Figs 4–6, Supplemental Digital Content 11, http://links.lww.com/IBD/B363. All markers are preselected based on the regional plots. The majority of selected variants are intergenic, intronic, or downstream and upstream variants of their respective genes with unknown functions. Genes selected with the regional plot are shown in brackets. These can mainly be linked to IBD-related diseases (CD and UC) and neurological diseases like Alzheimer's, autism and narcolepsy.

TABLE 4. Post Imputation Association Statistics of Markers with Genotypes; SNP with the Lowest *P*-value in ±400 kb Region

	CHR	ID	POS	REF	ALT	MA	MAF	R2	Beta (95% CI)	*P*
GP2 IgA (log10)	2	chr2:38392952	38392952	T	C	A	0.359	0.607	−0.114 (−0.169 to −0.060)	4.90×10^{-5}
	2	chr2:184086397	184086397	C	T	C	0.242	0.473	−0.155 (−0.233 to −0.077)	1.14×10^{-4}
	2	chr2:205829991	205829991	C	T	C	0.441	0.998	−0.110 (−0.165 to −0.054)	1.32×10^{-4}
	11	chr11:76334404	76334404	A	G	G	0.126	0.999	0.194 (0.114 to 0.274)	2.59×10^{-6}
GP2 IgG (log10)	2	chr2:103115860	103115860	C	G	A	0.059	1.001	−0.219 (−0.327 to −0.111)	7.81×10^{-5}
	3	chr3:33065715	33065715	C	G	G	0.019	0.732	0.435 (0.248 to 0.623)	6.59×10^{-6}
	12	chr12:78377963	78377963	T	C	A	0.081	0.238	0.284 (0.147 to 0.421)	5.55×10^{-5}
	13	chr13:44901189	44901189	G	A	A	0.073	0.997	0.232 (0.129 to 0.336)	1.33×10^{-5}
	19	chr19:51719479	51719479	A	T	C	0.426	0.577	−0.106 (−0.158 to −0.054)	6.70×10^{-5}
GP2 IgA (residuals)	2	chr2:38402571	38402571	T	A	A	0.367	0.738	−0.088 (−0.129 to −0.046)	4.85×10^{-5}
	2	chr2:184140295	184140295	C	T	C	0.297	0.285	0.103 (0.044 to 0.161)	5.83×10^{-4}
	2	chr2:205829991	205829991	C	T	C	0.441	0.998	−0.089 (−0.132 to −0.047)	4.63×10^{-5}
	11	chr11:76029879	76s879	T	C	G	0.488	0.673	−0.063 (−0.104 to −0.022)	3.05×10^{-3}

Positions are given in hg19; all calculations were performed on the ALT alleles.
ALT, alternative allele (according to imputation reference); CHR, chromosome; ID, sample identity number; MA, minor allele; MAF, minor allele frequency; POS, position; R2, imputation accuracy according to Michigan Imputation Server; REF, reference allele (according to imputation reference).

DISCUSSION

We tested 2 isoforms of 2 novel serum antibodies anti-GP2 IgA and IgG for diagnosis and differentiation of IBD as well as stratification and prediction of CD courses in a large, well-defined, and well-controlled German cohort. The tested serum markers were associated with CD and had a high discriminatory capability for CD versus UC. The immune response directed against GP2 isoform alpha was associated exclusively with stricturing disease behavior and need for IBD-related surgery. Anti-GP2 IgG was associated with ileal disease location. Interestingly, none of the antibodies was predictive of disease phenotypes. A GWA study of antibody expression in our cohort identified distinct loci associated with levels of anti-GP2 isoform beta IgG and IgA, many of which are known susceptibility loci for IBD.

PAB are known to be associated with CD.[15,17] Recently, glycoprotein 2 has been identified as the major target antigen of PAB.[18] It is not only present in pancreatic acinar cells but also in M cells of intestinal Peyer's patches. M cells are crucial in presenting luminal components to the underlying mucosa-associated immune system. Although direct proof is missing, this possibly explains its auto-antigenicity in patients with CD.[37] Mucosal GP2 mRNA is increased in antibody-positive patients with CD,[18] and release of GP2 secondary to intestinal damage could induce an antigenic response. M cells are abundant in the small intestine, in particular the ileum, and they are essentially absent in the large intestine.[38]

This could also explain the higher levels in CD subjects, in which 25% to 30% are positive for anti-GP2 IgG and/or IgA antibodies, whereas the frequency is lower in UC (9%–12%) or controls (0%–8%).[33,39] The link to disease location was confirmed in previous studies showing an association of anti-GP2 with ileal but not colonic CD.[19,20,32] Interestingly, anti-GP2 can also be detected in higher levels in the serum and feces of patients with ileal pouch–anal anastomosis with pouchitis than in normal pouch patients.[40] It, however, remains unclear why only a fraction of patients with CD loose tolerance and develop anti-GP2 antibodies and if these markers are an epiphenomenon of intestinal damage or a pathogenetic factor. GP2 recognizes FimH, expressed on the outer membrane of some enterobacilli, such as *Escherichia coli* and *Salmonella enterica*,[37] and facilitates phagocytosis. The presence of the respective FimH-positive strains in some but not other CD patients' microflora might provide a potential explanation for the subgroup of GP2-positive CD subjects. The fact that even anti-GP2-negative patients develop disease makes a direct pathogenetic relevance less likely. The accuracy of anti-GP2 for the discrimination of CD from all other groups was higher than ASCA. Although the low sensitivity of anti-GP2 indicates a limited clinical value for the primary diagnostic workup of CD, it could be useful after a thorough clinical workup in cases of indeterminate differentiation of CD versus UC.

Fibrostenosing complications in CD have been associated with a higher frequency of anti-GP2 isotype beta IgG in a Hungarian cohort,[26,32] and penetrating complications seem to have lower prevalence of anti-GP2 IgG. This specific association was confirmed by us but was not found by others.[21] We additionally tested the novel isoform alpha of anti-GP2 that had comparable accuracy in disease stratification. The ability to differentiate fibrostenosis from fistulizing disease is unique and has not been reported for ASCA that is elevated in both. There is agreement

between studies about a link of GP2 with need for surgical intervention.[19,21] This indicates that anti-GP2 could aid in the stratification of patients with IBD. It has to be mentioned that all but one study[21] are cross-sectional in design. GP2 IgA predicted a faster progression to first surgical resection in a univariate analysis, but this predictive capability got lost after correction for gender and clinical variables,[21] a finding that is comparable to our investigation in which none of the anti-GP2 antibodies, including its new isoform, are predictive of the later occurrence of complications.

Only one investigation to date assessed a possible link between a single gene and anti-GP2 expression in CD.[21] There was no association between anti-GP2 and *NOD2/CARD15* variants. In our GWA analysis, several candidates for an association of SNPs with anti-GP expression were identified. The majority of selected variants are intergenic, intronic, or downstream and upstream variants of their respective genes with unknown functions. The majority of the genes selected with the regional plots can be linked to IBD and neurological diseases. The variant with the strongest association is located approximately 34 kB downstream of *LRCCR32* with has been described, among others, in the context of IBD. This suggests that susceptibility loci for IBD lead to a stronger immune response towards GP2.

Antibody stability would be an important feature of a serologic marker. Pavlidis et al[19] reported that 3 of 8 seropositive patients with CD became negative over time, whereas 2 of 12 negative patients with CD turned positive during a median follow-up of 3 years. Papp et al[21] reported stability in antibody status in >95% of cases, and stability was not affected by the development of disease complications or the occurrence of surgery. GP2 is not the only identified antigen for the previously described anti-pancreas antibodies. The CUB and zona pellucida-like domains 1 (CUZD1) protein could be an alternate target. In an elegant study, Papp et al[21] showed that anti-CUZD1 is linked to CD and earlier development of perianal disease.

This study has several limitations: Our hospital is a regional referral center for severe CD cases introducing a selection bias. It would be desirable to test this antibody panel in a population-based cohort. This is reflected by the high frequency of patients with CD with complicated disease behavior and CD-related surgery. Not all patients who were assigned a behavioral disease phenotype according to the Montreal classification have the desired follow-up time of 5 years as suggested by Silverberg et al.[41] The sample size is robust but could be considered limited for the performance of a GWA study, and these results should be confirmed in independent studies.

CONCLUSIONS

The authors of this article believe that currently no serologic marker alone is accurate enough for the herein tested phenotypes, and a combination of markers and modalities shall be used for disease stratification and prediction. Anti-GP2 antibodies, including its isoform alpha, while carrying a high specificity, exhibit a rather low sensitivity for the diagnosis of CD. Anti-GP2 may be helpful in conjunction with other markers for disease stratification given the exclusive link with (but not prediction of) fibrostenotic disease phenotypes. A genetic association of IBD susceptibility SNPs with expression levels of anti-GP2 antibodies links disease pathogenesis with the formation of anti-pancreas antibodies.

ACKNOWLEDGMENTS

We would like to thank the physicians and study nurses of the Department of Internal Medicine I for their support of this study.

D. Roggenbuck is a shareholder of GA Generic Assays GmbH and Medipan GmbH. F. Rieder is on the Advisory Board at AbbVie and UCB and on the speakers' bureau for AbbVie. G. Rogler has consulted to Abbot, AbbVie, Augurix, Boehringer, Calypso, FALK, Ferring, Fisher, Genentech, Essex/MSD, Novartis, Pfizer, Phadia, Roche, UCB, Takeda, Tillotts, Vifor, Vital Solutions and Zeller; G. Rogler has received speaker's honoraria from AstraZeneca, Abbott, AbbVie, FALK, MSD, Phadia, Tillotts, UCB, and Vifor; G. Rogler has received educational grants and research grants from Abbott, AbbVie, Ardeypharm, Augurix, Calypso, Essex/MSD, FALK, Flamentera, Novartis, Roche, Takeda, Tillotts, UCB, and Zeller. The remaining authors have no conflict of interest to disclose.

REFERENCES

1. Schoepfer AM, Dehlavi MA, Fournier N, et al. Diagnostic delay in Crohn's disease is associated with a complicated disease course and increased operation rate. *Am J Gastroenterol.* 2013;108:1744–1753; quiz 1754.
2. Dignass A, Lindsay JO, Sturm A, et al. Second European evidence-based consensus on the diagnosis and management of ulcerative colitis part 2: current management. *J Crohns Colitis.* 2012;6:991–1030.
3. Dignass A, Van Assche G, Lindsay JO, et al. The second European evidence-based consensus on the diagnosis and management of Crohn's disease: current management. *J Crohns Colitis.* 2010;4:28–62.
4. Thia KT, Sandborn WJ, Harmsen WS, et al. Risk factors associated with progression to intestinal complications of Crohn's disease in a population-based cohort. *Gastroenterology*. 2010;139:1147–1155.
5. Peyrin-Biroulet L, Loftus EV Jr, Colombel JF, et al. The natural history of adult Crohn's disease in population-based cohorts. *Am J Gastroenterol.* 2010;105:289–297.
6. Devlin SM, Dubinsky MC. Determination of serologic and genetic markers aid in the determination of the clinical course and severity of patients with IBD. *Inflamm Bowel Dis.* 2008;14:125–128; discussion 132–123.
7. Targan SR. The utility of ANCA and ASCA in inflammatory bowel disease. *Inflamm Bowel Dis.* 1999;5:61–63; discussion 66–67.
8. Sutton CL, Kim J, Yamane A, et al. Identification of a novel bacterial sequence associated with Crohn's disease. *Gastroenterology*. 2000;119:23–31.
9. Ferrante M, Henckaerts L, Joossens M, et al. New serological markers in inflammatory bowel disease are associated with complicated disease behaviour. *Gut*. 2007;56:1394–1403.
10. Papp M, Altorjay I, Dotan N, et al. New serological markers for inflammatory bowel disease are associated with earlier age at onset, complicated disease behavior, risk for surgery, and NOD2/CARD15 genotype in a Hungarian IBD cohort. *Am J Gastroenterol.* 2008;103:665–681.
11. Targan SR, Landers CJ, Yang H, et al. Antibodies to CBir1 flagellin define a unique response that is associated independently with complicated Crohn's disease. *Gastroenterology*. 2005;128:2020–2028.

12. Mow WS, Vasiliauskas EA, Lin YC, et al. Association of antibody responses to microbial antigens and complications of small bowel Crohn's disease. *Gastroenterology*. 2004;126:414–424.
13. Vasiliauskas EA, Kam LY, Karp LC, et al. Marker antibody expression stratifies Crohn's disease into immunologically homogeneous subgroups with distinct clinical characteristics. *Gut*. 2000;47:487–496.
14. Landers CJ, Cohavy O, Misra R, et al. Selected loss of tolerance evidenced by Crohn's disease-associated immune responses to auto- and microbial antigens. *Gastroenterology*. 2002;123:689–699.
15. Klebl FH, Bataille F, Huy C, et al. Association of antibodies to exocrine pancreas with subtypes of Crohn's disease. *Eur J Gastroenterol Hepatol.* 2005;17:73–77.
16. Koutroubakis IE, Drygiannakis D, Karmiris K, et al. Pancreatic autoantibodies in Greek patients with inflammatory bowel disease. *Dig Dis Sci.* 2005;50:2330–2334.
17. Seibold F, Weber P, Jenss H, et al. Antibodies to a trypsin sensitive pancreatic antigen in chronic inflammatory bowel disease: specific markers for a subgroup of patients with Crohn's disease. *Gut*. 1991;32:1192–1197.
18. Roggenbuck D, Hausdorf G, Martinez-Gamboa L, et al. Identification of GP2, the major zymogen granule membrane glycoprotein, as the autoantigen of pancreatic antibodies in Crohn's disease. *Gut*. 2009;58:1620–1628.
19. Pavlidis P, Romanidou O, Roggenbuck D, et al. Ileal inflammation may trigger the development of GP2-specific pancreatic autoantibodies in patients with Crohn's disease. *Clin Dev Immunol.* 2012;2012:640835.
20. Somma V, Ababneh H, Ababneh A, et al. The novel Crohn's disease marker anti-GP2 antibody is associated with ileocolonic location of disease. *Gastroenterol Res Pract.* 2013;2013:683824.
21. Papp M, Sipeki N, Tornai T, et al. Rediscovery of the anti-pancreatic antibodies and evaluation of their prognostic value in a prospective clinical cohort of Crohn's patients: the importance of specific target antigens [GP2 and CUZD1]. *J Crohns Colitis.* 2015;9:659–668.
22. Roggenbuck D, Reinhold D, Wex T, et al. Autoantibodies to GP2, the major zymogen granule membrane glycoprotein, are new markers in Crohn's disease. *Clin Chim Acta.* 2011;412:718–724.
23. Pavlidis P, Komorowski L, Teegen B, et al. Diagnostic and clinical significance of Crohn's disease-specific pancreatic anti-GP2 and anti-CUZD1 antibodies. *Clin Chem Lab Med.* 2016;54:249–256.
24. Michaels MA, Jendrek ST, Korf T, et al. Pancreatic autoantibodies against CUZD1 and GP2 are associated with distinct clinical phenotypes of Crohn's disease. *Inflamm Bowel Dis.* 2015;21:2864–2872.
25. Pavlidis P, Forbes A, Bogdanos DP. Anti-GP2 antibodies in inflammatory bowel disease patients with ileal pouch. *J Crohns Colitis.* 2013;7:e602–e603.
26. Pavlidis P, Forbes A, Bogdanos DP. Antibodies to glycoprotein 2 (GP2) in patients with inflammatory bowel diseases from UK. *Clin Chim Acta.* 2011;412:1163–1164.
27. Papp M, Norman GL, Vitalis Z, et al. Presence of anti-microbial antibodies in liver cirrhosis–a tell-tale sign of compromised immunity? *PLoS One*. 2010;5:e12957.
28. Sakly W, Jeddi M, Ghedira I. Anti-Saccharomyces cerevisiae antibodies in primary biliary cirrhosis. *Dig Dis Sci.* 2008;53:1983–1987.
29. Deleted in proof.
30. Roggenbuck D, Reinhold D, Hiemann R, et al. Standardized detection of anti-ds DNA antibodies by indirect immunofluorescence—a new age for confirmatory tests in SLE diagnostics. *Clin Chim Acta.* 2011;412:2011–2012.
31. Roggenbuck D, Rober N, Bogdanos DP, et al. Autoreactivity to isoforms of glycoprotein 2 in inflammatory bowel disease. *Clin Chim Acta.* 2015;442:82–83.
32. Bogdanos DP, Roggenbuck D, Reinhold D, et al. Pancreatic-specific autoantibodies to glycoprotein 2 mirror disease location and behaviour in younger patients with Crohn's disease. *BMC Gastroenterol.* 2012;12:102.
33. Roggenbuck D, Reinhold D, Schierack P, et al. Crohn's disease specific pancreatic antibodies: clinical and pathophysiological challenges. *Clin Chem Lab Med.* 2014;52:483–494.
34. Rieder F, Schleder S, Wolf A, et al. Association of the novel serologic anti-glycan antibodies anti-laminarin and anti-chitin with complicated Crohn's disease behavior. *Inflamm Bowel Dis.* 2010;16:263–274.
35. Rieder F, Schleder S, Wolf A, et al. Serum anti-glycan antibodies predict complicated Crohn's disease behavior: a cohort study. *Inflamm Bowel Dis.* 2010;16:1367–1375.
36. Vermeire S, Peeters M, Vlietinck R, et al. Anti-Saccharomyces cerevisiae antibodies (ASCA), phenotypes of IBD, and intestinal permeability: a study in IBD families. *Inflamm Bowel Dis.* 2001;7:8–15.
37. Hase K, Kawano K, Nochi T, et al. Uptake through glycoprotein 2 of FimH(+) bacteria by M cells initiates mucosal immune response. *Nature*. 2009;462:226–230.
38. Kraehenbuhl JP, Neutra MR. Epithelial M cells: differentiation and function. *Annu Rev Cell Dev Biol.* 2000;16:301–332.
39. Bogdanos DP, Rigopoulou EI, Smyk DS, et al. Diagnostic value, clinical utility and pathogenic significance of reactivity to the molecular targets of Crohn's disease specific-pancreatic autoantibodies. *Autoimmun Rev.* 2011;11:143–148.
40. Werner L, Sturm A, Roggenbuck D, et al. Antibodies against glycoprotein 2 are novel markers of intestinal inflammation in patients with an ileal pouch. *J Crohns Colitis.* 2013;7:e522–e532.
41. Silverberg MS, Satsangi J, Ahmad T, et al. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. *Can J Gastroenterol.* 2005;19(suppl A):5A–36A.